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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Paul R. Schimmel

Serial No.: 08/249,689 Art Unit: 1631

Filed: May 26, 1994 Examiner: J. Brusca

For: *"DESIGNING COMPOUNDS SPECIFICALLY INHIBITING RIBONUCLEIC ACID FUNCTION"*

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I, James R. Williamson, hereby declare that:

1. I am a Professor of Molecular Biology and Chemistry at The Scripps Research Institute in La Jolla, CA, and hold a Ph.D. in Chemistry from Stanford. I have over 14 years experience in the field of RNA structure, RNA-protein recognition, and RNA-small molecule. This includes specific experience in binding of compounds to RNA. A partial curriculum vitae is attached to this declaration as an exhibit.

2. I have reviewed the specification of the above-identified application, and the claims as filed July 2, 2001.

3. I have reviewed the Office Action mailed January 11, 2002, in connection with the above-identified application.

4. I understand that claims 11-13, 17-19, and 21 define a compound comprising hydrogen bond donor and acceptor sites arranged to specifically bind and inhibit the function of

a targeted RNA molecule, wherein the compound is specifically directed to and binds to a critical region of the RNA molecule, located within the minor groove of the RNA molecule, identified by a combination of the primary, secondary and tertiary structure of the critical region. It is my further understanding that these claims have been rejected on the basis that one skilled in the art in September 1990, would not know the structure or identity of these compounds as defined by the claims, based on the specification and what was known to those skilled in the art at the time. This rejection is referred to as a failure to comply with the written description requirement. I have been advised that to comply with the written description, one must meet the following legal standard:

> "....conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it. Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it." *Amgen*, 927 F.2d at 1206, 18 USPQ 2d at 1021. Furthermore the court has stated that in order to satisfy the written description requirement, "the applicant need not describe the subject matter claimed in exact terms. However, the description must clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed." *Monsanto Co. v. Mycogen Plant Science*, Inc., 61 F.Supp.2d 133, 188 (D.Del. Aug 18, 1999).

5. As an expert in the field of RNA and drug design in general, and as an individual with extensive knowledge of the level of understanding of those of skill in the art as of September

1990, I believe that the specification and claims, in view of what was known to those in the art, provides a written description that is sufficient to comply with the legal standard as defined above. I present in this declaration evidence indicating that attractive and repulsive forces present in the critical region of the minor groove of RNA dictate or define the geometrical constraints of the region. These forces, as described in the specification, and below, define the structure of the critical region in a way that provides one with a mental picture of a defined "space" that can only be accessed by a compound of the correct "shape". It will help, perhaps, to view the minor groove as a "lock" and the compound as the "key", wherein the shape of the interior of the lock is defined by hydrophobic, hydrogen bonding, and electrostatic forces provided by nucleic acid bases. The key (compound) will only fit into the lock if it is able to "complement" these forces.

6. RNA usually forms double stranded regions by looping and base pairing complementary, palindromic and/or nearly palindromic sequences. Double stranded helical regions will form the major and minor grooves often used in the geometric descriptions of nucleic acid molecules. RNA geometry may deviate from what is usually defined as Watson-Crick base pairing, which lies at the core of normal DNA helix formation. However, RNA geometries such as symmetrical or asymmetric interior loops, bulge loops, purine-purine mispairs, GU or wobble pairs and pseudoknots (which are tandem stem loops) all retain the ability to form major and minor grooves. Such geometrical arrangements provide unique platforms for binding of compounds in the minor groove.

The critical region in the minor groove as described in the specification is sufficient to describe the claimed inhibitory compound. The primary basis for sequence discrimination in RNA is the minor groove. Nucleotide bases and sequences of bases are most accessible to compounds via the minor groove. The minor groove is not only wider, but significantly more shallow than the relatively narrower and deeper groove provided by the major groove. The major groove is too deep and too narrow for a compound to make direct sequence specific contact. The nature of the bases recognized by any particular amino acid side chain or other compound depends upon the local geometry within the minor groove. Factors determining the geometric configuration of the critical region of the minor groove are the hydrophobicity of the local environment, the pattern of accessible hydrogen bond donors and acceptors present, and the repulsive and attractive forces that exist as electrostatic entities within the targeted minor groove.

Hydrophobic Environment of the Minor Groove

Planar aromatic purines and pyrimidines interact strongly to form parallel stacked structures. As stated at page 2, lines 21-29 of the specification, the nucleotide bases of the RNA molecule are planar and perpendicular to the helical axis. Because the RNA helix is in an alpha conformation, the bases and sequences of bases are most accessible from the minor groove. Energetically speaking, such stacked structures are critical in determining the nucleic acid conformation. Stacked structures tend to be maximized in nucleic acids. For example, base stacking occurs in the tRNA structure to almost the maximum conceivable extent.

A consequence of the extensive stacking and base pairing is that many of the bases are rendered inaccessible to solvent. Inaccessibility to solvent provides for a local hydrophobic and

nonaqueous environment. Taken together with the fact that apolar side-chain moieties of amino acids will prefer to reside in an apolar nonaqueous environment, one will realize that the extensive stacking of bases provides an ideal hydrophobic environment for compounds that exhibit apolar-like surfaces to bind. Many of these surfaces will become exposed once initial contact is made with the target RNA (many times this contact will induce a conformational change wherein the binding conformation of the compound is stabilized). An example of a motif in which apolar residues *are exposed* is the RNA Recognition Motif, or RRM, found in many RNA binding proteins. RNA binds on the flat face of a β-sheet in RRM, which carries a number of Arg, Lys and sometimes His residues. Residues such as these provide electrostatic interactions with the RNA backbone. The non-polar aromatic residues are ideally located, exposed on the face of the β-sheet, where they can interact and stack with bases of the RNA.

Hydrogen Bonding

The chemical basis for the discrimination between different base pairs lies in the order of hydrogen bond acceptor and donor groups across the base pair that is accessible to a particular compound. Thus, the compound/base pair specific interaction is part of a network of specific hydrogen bonds. Page 7, lines 24-26, teach this point as it relates to the minor groove. Because there are actually fewer differences in the pattern of potential hydrogen bond donors and acceptors in the minor groove (G:C and A:U), the specific pattern of H-bond acceptors and donors that may be present on a specific inhibitory compound is further limited by the minor groove. Single stranded nucleic acid is more flexible and can twist and turn to meet the compound's hydrogen bonding pattern, thereby making it extremely difficult to meet the

specificity requirements of the compound. However, those RNA molecules, such as those described above and in the specification, are less flexible because of their formation of secondary structures, such as base pairing. Base paired regions also limit the flexibility in the nearby single stranded regions. Less flexibility will provide opportunity for *specific* interactions via a hydrogen bond network that is static, not in a state of flux.

Electrostatic Interactions

The electrostatic *force* between the compound and the targeted RNA defines the *affinity* of the interaction. The hydrophobic interactions and hydrogen bonds, described above, are short range interactions based on induced-dipole and molecular dipole moments. Because electrostatic interactions can be sensed several angstroms from the point charge, they are considered to be long range interactions. The strength of these electrostatic interactions is a function of the dielectric property of the local environment. Chemical side groups on the inhibitory compound, provided that they are in the correct spatial location, orientation, and have the correct charge, will increase the strength of these electrostatic interactions. A higher degree of complementarity will strengthen these type of interactions. Such complementarity is achieved via the maximization of hydrogen bonding, hydrophobic interactions, and defining the size of the compound based upon the known dimensions of the targeted minor groove.

Geometric and Steric constraints

Given that the minor groove of RNA is derived from a combination of double stranded regions of nucleic acid structure, the arrangement of the nucleotide bases of the RNA molecule that are planar and perpendicular to the helical axis, the formation of a hydrophobic core, as well

as the hydrogen bonding network present as "acceptors" and "donors", the field of RNA biochemistry realizes that these constraints provide a very defined geometry that is present within the minor groove. While the minor groove is wider and more shallow than its counterpart, the major groove, the minor groove can be defined geometrically and sterically because of the hydrophobic nature, hydrogen bonding, and electrostatic forces that are present. All of these "constraints" define the nature of the inhibitory compound in terms of structure and functionality.

As described at pages 19 and 20 of the specification, what was known in the prior art clearly established the importance of hydrogen bonding and hydrophobic interactions in the recognition of specific DNA sequences by proteins such as repressors and endonucleases. Studies such as these have provided the impetus for understanding the helical configuration of RNA, and more specifically, the minor groove. Again, what is critical is the correct spatial arrangement of hydrogen bond donors and acceptors on the inhibitor, as well as the correct geometrical shape as defined by the width, depth, and bonding forces present in the minor groove. The specification defines the forces, as further described above, that establish the structure of the critical region in terms of specific available interactions and geometry. These features are easily obtained upon identifying the RNA sequence to be targeted. Secondary and tertiary structures of the targeted RNA can be derived from any number of commercially available programs. Such programs include AMBER (Assisted Model Building with Energy Refinement, developed at UCSF), CHARMM (developed at Harvard), MFOLD (prediction of RNA secondary structure by Energy Minimization), RNAfold (calculate secondary structures of

RNAs), RNAeval (calculate energy of RNA sequences on given secondary structure), RNAheat (calculate specific heat of RNAs), RNAdistance (calculate distances of RNA secondary structures), RNApdist (calculate distances of thermodynamic RNA secondary structures ensembles), RNAinverse (find RNA sequences with a given secondary structure), RANsubopt (calculate suboptimal secondary structures of RNAs), palindrome (identify inverted repeats in a nucleotide sequence), and RNAGA (predict common secondary structures of RNAs by genetic algorithm).

7. There is precedent for recognition of a groove with a designed ligand from the study of DNA. The sequence of DNA is sufficient to describe the nature of a compound that will bind specifically to DNA in the minor groove, as described by Peter Dervan at Caltech (Dervan PB. "Design of sequence-specific DNA-binding molecules." *Science. 232*, p. 464-71, (1986). The helical geometry of DNA is B-form, which is distinct from the A-form helical geometry found for RNA in terms of helical pitch, as well as the size and shape of the major and minor grooves. Furthermore, in RNA-helices, G-U wobble pairs are often present that present specific groups in the minor groove that provide unique features for recognition.

8. There is precedent for formation of a complementary interface for binding to the minor groove of RNA. The binding of ribosomal protein S15 to a fragment of ribosomal RNA demonstrates that presentation of a suitable array of hydrogen bonding and electrostatic groups by a molecule that is complementary to the minor groove allows specific recognition. The structure of S15 bound to RNA was solved in our laboratory (Sultan C. Agalarov, G. Sridhar Prasad, Peter M. Funke, C. David Stout, and James R. Williamson, "Structure of the S15, S6,

S18-rRNA Complex:Assembly of the 30 S Ribosome Central Domain", *Science 288,*107-112 (2000).) The S15 protein interacts with two successive minor grooves separated by a turn of helix. In the first turn, a tandem pair of G-C, C-G base pairs is recognized, while in the second turn, a tandem pair of G-U, G-C is recognized. A number of side chains make specific contacts with the functional groups displayed in the minor groove of the A-form RNA, demonstrating that a complementary interface can be formed. Simply identifying the sequence of the nucleotides in the groove implies the specific conformation, and defines the array of complementary groups that must be assembled in order to recognize that sequence. A figure is shown with the interactions of the S15 protein with RNA (Figure 1, attached).

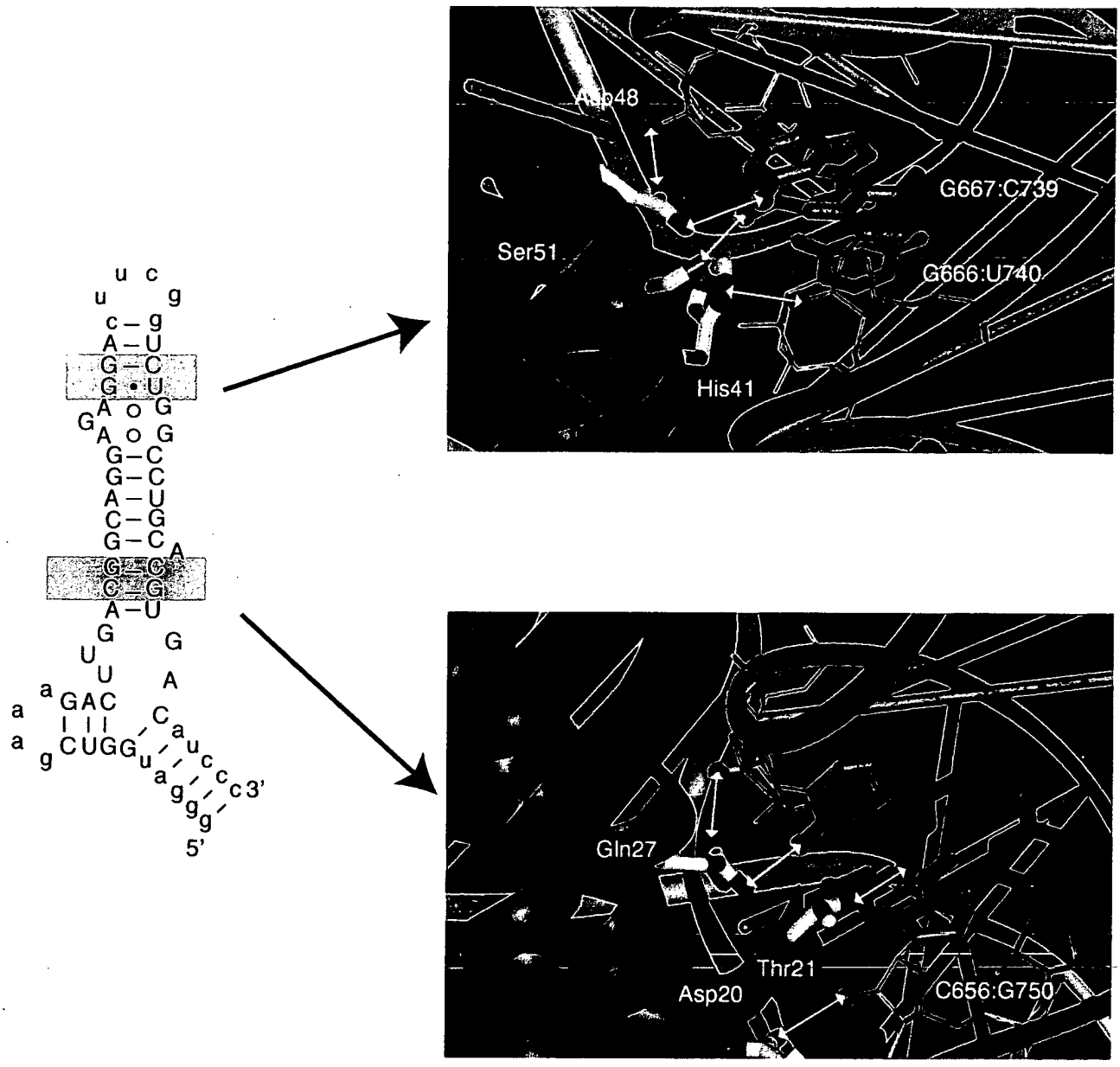
9. I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 3/10/02

James R. Williamson

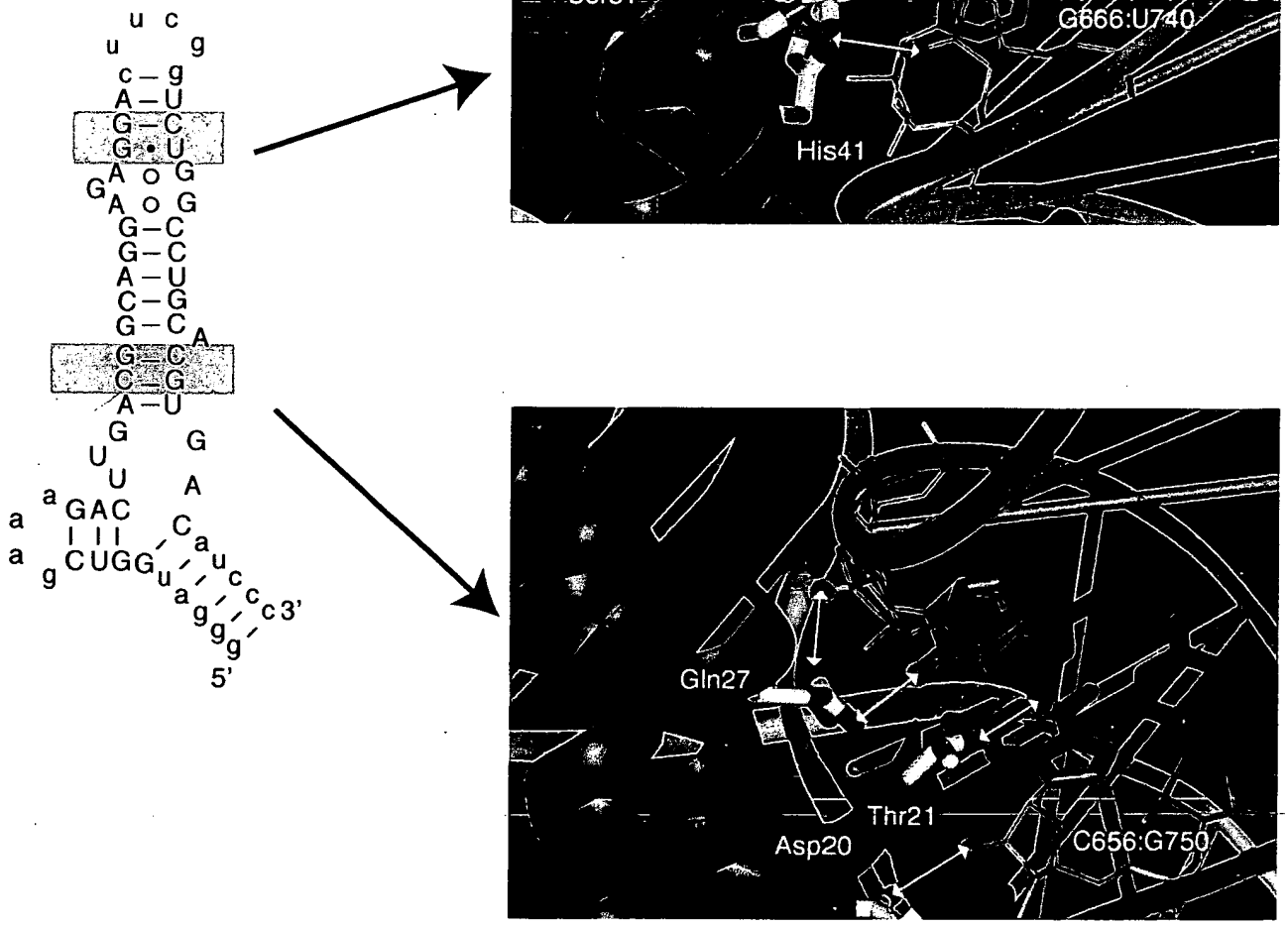
ATL1 #506069 v1

Figure 1: RNA minor groove recognition by protein S15



Legend: At left is shown the RNA binding site for the protein S15. The RNA structure consists of A-form helices joined by bulge and loop segments. S15 recognizes two distinct regions in the minor groove, and the structure of those regions is shown at right. S15 is shown in red, and the RNA is shown in cyan. The two base pairs that are recognized are shown in green and magenta. In the upper panel, successive G-U and G-C base pairs are recognized bye three side chains on S15. In the lower panel, successive C-G and G-C base pairs are recognized by three side chains on S15.

Figure 1: RNA minor groove recognition by protein S15



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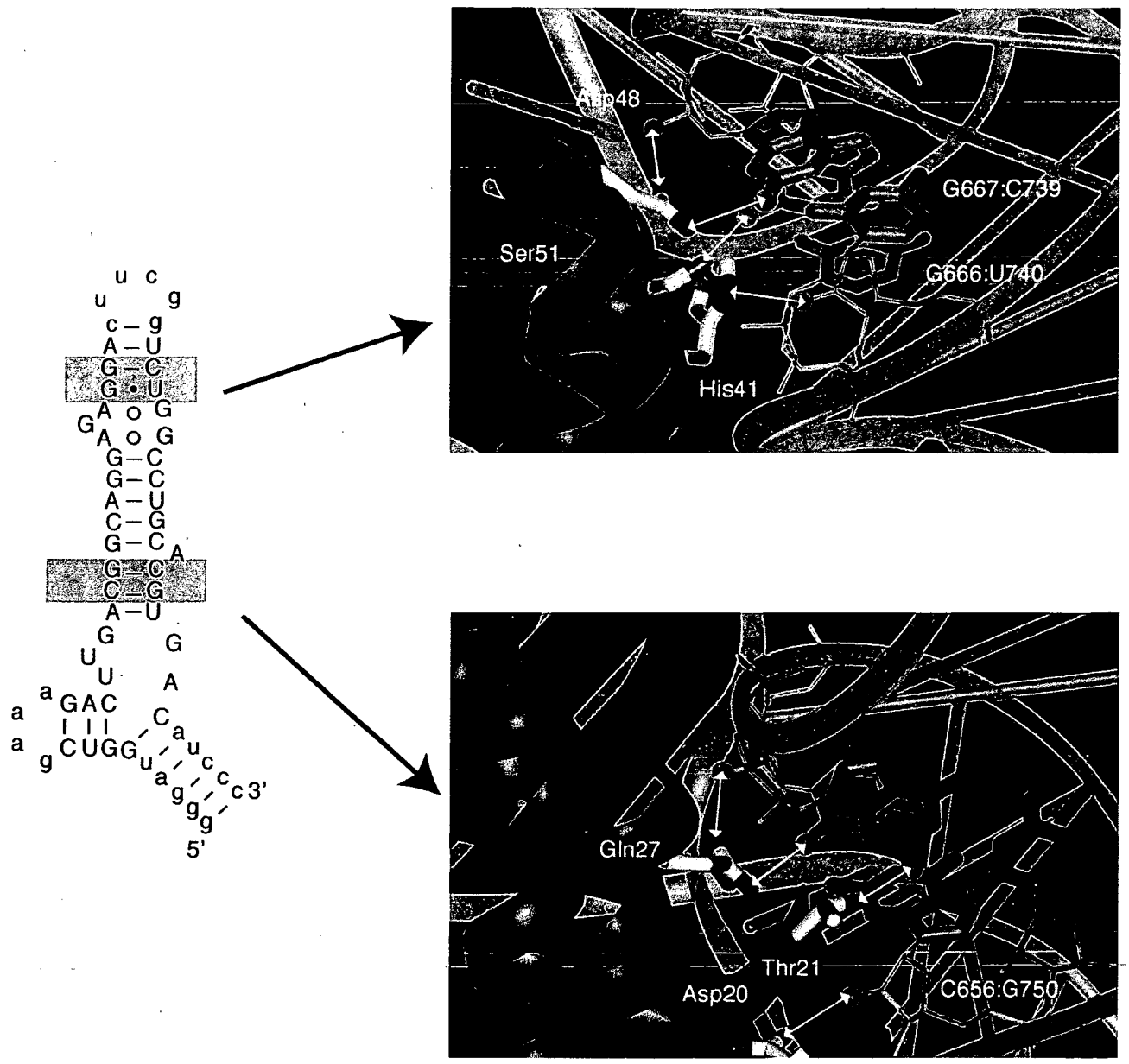

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